Erratum

Erratum to: Gentamicin Reduces Calcific Nodule Formation by Aortic Valve Interstitial Cells *In Vitro*

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We regret that this paper was published with some minor errors. The corrected Figures 2–5 are presented here along with the corresponding text from the results section that describes these data.

RESULTS

Effects of Gentamicin on Cellular Mineralization

For the ARS assay, there was a significant reduction in the number of nodules with every increasing concentration of gentamicin. In addition, there was a significant increase in the average size per nodule for the 0.1 and 0.2 mM gentamicin groups compared to control (representative nodules shown in Figs. 1a, 1b). The overall decrease in nodule number and increase in nodule size from the control group to the 0.2 mM gentamicin group was 49 and 57%, respectively (Figs. 2a, 2b). The total calcified area was significantly reduced for the 0.05, 0.1, and 0.2 mM gentamicin groups, resulting in 21% less calcified area at the highest gentamicin concentration of 0.2 mM compared to control (Fig. 2c).

Similar to the ARS assay, the von Kossa staining showed a significant decrease in nodule number at every increasing concentration of gentamicin and a significant increase in nodule size for the 0.1 and 0.2 mM gentamicin groups compared to control (data not shown; representative nodules shown in Figs. 1c–1d). The overall decrease in nodule number and increase in nodule size from the control group to the 0.2 mM gentamicin group was 47 and 51%, respectively. The total calcified area also decreased with

gentamicin concentration, with the 0.2 mM gentamicin group having 20% less calcified area than the control group (Fig. 3a). The same pattern was shown by the alkaline phosphatase assay, with the 0.2 mM gentamicin group having 49% less alkaline phosphatase compared to control (Fig. 3b). For both von Kossa total calcified area and the alkaline phosphatase assay, the 0.1 and 0.2 mM gentamicin groups were significantly different from controls.

Effect of Gentamicin of Cellular Proliferation and Apoptosis

There was a significant increase in cell number for the 0.1 and 0.2 mM gentamicin groups compared to control ($p < 0.05$), with a 47% increase in cell number for the 0.2 mM gentamicin group compared to control (Fig. 4a). Similarly, there was a significant increase in the content of caspases 3 and 7 for the 0.1 and 0.2 mM gentamicin groups, with a 78% increase in caspase content in the 0.2 mM gentamicin group compared to control (Fig. 4b).

Effects of Gentamicin on Previously Calcified Cell Cultures

There were two control groups for this experiment. The first control was the initial control group, which had been previously calcified by β -glycerophosphate treatment for 8 days. In addition, a final control 16 day group was included, in which the samples were allowed to calcify endogenously, without β -glycerophosphate or gentamicin, for an additional 8 days. By all measures, the final control group was significantly more calcified than the initial control group (Figs. 5, 6).

The ARS assay showed that treatment every day with gentamicin reduced the number of nodules and total calcified area compared to the 16 day control. Interestingly, treatment every day reduced the total calcified area and nodule number to 27 and 31%, respectively, less than the initial 8-day control

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FIGURE 2. Use of the Alizarin Red S stain showed that gentamicin concentration dose-dependently reduced nodule number (a), increased nodule size (b), and reduced the total calcified area (c). Groups not connected by same letter are significantly different ($p < 0.05$).

FIGURE 3. Use of the von Kossa stain showed that gentamicin dose-dependently reduced the total calcified area (a) and alkaline phosphatase content (b) of the pAVIC cultures. Groups not connected by same letter are significantly different ($p < 0.05$).

FIGURE 4. Use of MTT assay and Caspase 3/7 assay showed that gentamicin dose-dependently increased cellular number (a) and apoptosis (b). Groups not connected by same letter are significantly different ($p < 0.05$).

FIGURE 5. The application of gentamicin to previously calcified pAVIC cultures reduced the number of calcium-containing nodules (a) and the total calcified area (b), as demonstrated by Alizarin Red S, depending upon the frequency of treatments. Groups not connected by same letter are significantly different.

(Figs. 5a, 5b). Treatment for only one day, in contrast, reduced nodule number below the final 16 day control but there was no significant difference in total calcified area between one day and the final 16 day control. The groups in which gentamicin was added for 4 days total (in different application patterns) were not significantly different from each other for nodule number, but the every 2 days group was significantly different from the every other day group for total calcified area. In addition, all had lower nodule number and total calcified area compared to the final 16 day control values. The two groups that had gentamicin applied for 2 consecutive days both had fewer nodules than in the initial 8 day control. There was no effect of treatment pattern on average nodule size.